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1996

Status Report

Stored-Product Insects Research of of Military Importance

Biological Research Unit
U. S. Grain Marketing Research Laboratory
Grain Marketing and Production Research Center

1515 College Avenue
Manhattan, Kansas 66502

1996 Status Report

"Stored-Product Entomology Research"

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U. S. GRAIN MARKETING RESEARCH LABORATORY

GRAIN MARKETING AND PRODUCTION RESEARCH CENTER

LABORATORY MISSION

The mission of the U. S. Grain Marketing and Research Laboratory is to develop new knowledge, information, and technologies needed to solve problems associated with the harvest, storage, marketing, and overall quality and end-use properties of cereal grains. The Laboratory program focuses on post-harvest aspects of grain marketing and includes a major commitment to incorporation of optimum end-use quality characteristics into new grain varieties in cooperation with plant breeders. The Laboratory provides multidisciplinary research on these issues by three research units:

Biological Research Unit
Engineering Research Unit
Grain Quality and Structure Research Unit

MISSION OF THE BIOLOGICAL RESEARCH UNIT

The mission of the Biological Research Unit is to develop new, ecologically-based methods to replace or reduce the use of traditional pesticides for controlling insects in stored grain and stored products. A multidisciplinary team conducts research on 1) novel physiological, genetic, and biological control techniques; 2) improved insecticide deployment and resistance management strategies; 3) the ecology population dynamics, and behavior of pest and beneficial insects; and 4) the development of computer-based integrated pest management systems for farm and commercial grain storage and processing facilities.

We conduct research on biological control agents, insect-resistant packaging, novel physiological control techniques, host plant resistance, insecticide deployment and resistance management strategies, insect biochemistry and genetics, insect population monitoring, population dynamics and behavior, and computer-based integrated pest management systems.

BIOLOGICAL RESEARCH UNIT

U.S. GRAIN MARKETING RESEARCH LABORATORY

STAFFING/AREA OF RESEARCH EMPHASIS

Research Leader

Dr. William H. McGaughey, Supervisory Res. Entomologist	Microbial Insecticides, Integrated Pest Management

Scientists

Dr. Franklin H. Arthur, Res. Entomologist	Integrated Pest Management Technologies
Dr. James E. Baker, Res. Entomologist	Parasitoid Biology and Toxicology; Digestive Physiology
Dr. Richard W. Beeman, Res. Entomologist	Genetics/Molecular Biology; Insecticide Resistance
Dr. Charles S. Burks, Res. Ecologist	Cold Temperature Insect Behavior
Dr. Alan K. Dowdy, Res. Entomologist	Insect Ecology/Behavior in Commercial Facilities
Dr. Paul W. Flinn, Res. Biologist	Modeling/Expert Systems
Dr. David W. Hagstrum, Res. Entomologist	Insect Ecology, Modeling, Sampling, Acoustic Detection
Dr. Ralph W. Howard, Res. Chemist	Chemical Ecology; Biological Control
Dr. Donovan E. Johnson, Res. Microbiologist	Microbiology of Insect Pathogens
Dr. Karl J. Kramer, Res. Chemist	Insect Biochemistry and Physiology, Biopesticides
Dr. Michael A. Mullen, Res. Entomologist	Insect Trapping; Insect Resistant Packaging
Dr. Brenda S. Oppert, Res. Biologist	Biochemistry, B.t. Research
Dr. James E. Throne, Res. Entomologist	Ecology, Modeling, Seed Resistance to Insects
Dr. Yu-Cheng Zhu, Res. Entomologist	Molecular Biology

RESEARCH HIGHLIGHTS AND TECHNOLOGY TRANSFER

Some *Bacillus thuringiensis* (b.t.) resistant strains of *P. interpunctella* were found to lack a major gut proteinase. The linkage between the absence of a major gut proteinase and Bt resistance was suggested by genetic crosses between Bt susceptible and resistant insects.

CO_2 insect detection was shown to be a feasible alternative to X-ray detection of insects infesting spices.

Malathion resistance in the parasitic wasp, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) was determined to be inherited as an incompletely dominant trait controlled by a single gene.

Insects pay a price for resistance. Natural mortality among Indianmeal moth larvae with resistance to *Bacillus thuringiensis* was higher than in larvae that lacked *B. thuringiensis* resistance. The acquisition of *B. thuringiensis* resistance in these insects resulted in reduced fitness, which may affect their ability to compete and survive in the field.

Host-parasitoid interactions studied. A detailed behavioral analysis of how the parasitoid, *Cephalonomia tarsalis*, locates, recognizes and parasitizes its host, the saw-toothed grain beetle was conducted. This is an important step toward using this parasitoid for biological control of stored product insects.

Semiochemical-behavioral relationship studied. Cuticular lipids of the parasitoid *Pteromalus cereallallae* have been characterized. The males and females have distinctly different profiles. Experiments are in progress to test the roles of these lipids in gender recognition, and possible use in courtship behavior.

Insect hormones regulate insect immune responses. Insect hormones known as eicosanoids were shown to be important regulators of the ability of a stored grain feeding beetle to mount an immune response against pathogenic bacteria. The insect was shown to contain the necessary precursor fatty acids for producing these hormones in all life stages. These findings are an important step in devising strategies to hinder the ability of stored grain insect pests to resist microbial biocontrol treatments.

Determined crystallization temperature and 2-h lethal temperature thresholds for the rice weevil, the red flour beetle, the saw-toothed grain beetle, and the lesser grain borer. Also obtained these data for three parasitoids (*Anisopteromalus calandrae, Cephalonomia tarsalis,* and *Choetospila elegans*) which attack these coleopteran species. These data provide absolute lethal thresholds for these species, and will allow us to distinguish chilling and freezing injury in subsequent studies.

Cold hardiness documented in overwintering face flies, *Musca autumnalis.* Crystallization temperature and chilling injury data were obtained for the house fly, *Musca domestica*. While this work is primarily intended to develop these prolific, short-lived species as a model system for mechanisms of chilling injury and acclimation, it can also suggest management practices for these medical and veterinary pests in temperate climates.

Population growth model for lesser grain borer improved. New data on lesser grain borer developmental time, reproduction and survival were used to improve model predictions. Predictions were validated over a broad range of grain moisture and temperature conditions.

A rapid microplate assay for the analysis of mixtures of insect enzymes was developed. The technique uses r-nitroanilide substrates. This technique is applicable in a wide range of insect physiology studies.

Knowledge of novel insect enzyme genes may lead to more effective pest management. Certain enzymes that are selectively toxic to insects are being developed by ARS as biopesticides for insect control purposes. A gene for an insect molting enzyme has been cloned and characterized by ARS and Kansas State University scientists. This enzyme degrades the protective linings of the insect's gut and exoskeleton and is toxic when fed to insects. A seed company has been licensed by ARS and Kansas State Univesity to evaluate this gene for resistance to pest insects when it is expressed by transgenic plants. This collaboration is a critical step in the commercial development of this transgene as a biopesticide for many types of agricultural pest insects.

US and foreign patent applications for the use of insect chitinase as a biopesticide filed. ARS scientists at Manhattan, Kansas, in cooperation with scientists at Kansas State University, have been working to enhance the resistance of plants to insects using chemical defense transgenes. The gene for an insect molting enzyme can be manipulated by the agricultural biotechnology industry for the improvement of host plant resistance to insect pests. Patent applications for the use of insect chitinase as a biocide in the US and several foreign countries were filed.

Knowledge of insect skeletal structures may lead to more effective pest Management. The insect exoskeleton is a good target for new pest management strategies because of the novel and unique insect-specific chemistry that occurs during its formation. However, development of exoskeleton-targeted insect control agents has been hampered by a lack of basic knowledge about insect skeletal structure and metabolism. ARS and university scientists have identified novel types of chemical bonding in the exoskeleton, as well as several enzymes that catalyze the formation of these bonds. Inhibition of these enzymes by biopesticides may be an environmentally-safe method of insect pest control.

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-014-00D

Title: Ecology, Modeling, and Integrated Management of Stored-Product Insects

Main Objectives:

Pest management decisions can be improved by developing better insect monitoring programs and models for predicting insect population growth. Research will provide the technology that grain managers need to detect insect infestations more accurately and earlier, predict insect population growth rates and effects of different control measures, and forecast when insect control will be needed. These management tools are needed for a transition to less chemically dependent integrated pest management programs. This project focuses on acquiring essential biological and ecological data, using these data to develop predictive models of insect population growth, and developing an expert system for stored grain management that uses the models. Studies will expand the range of species and conditions over which existing models predict insect population growth and the range of problems for which the expert system can make pest management recommendations. Expansions will include additional pest species, natural enemies, grain varieties, aeration, cold temperature survival, and insect movement. Improved sampling programs will be developed to collect ecological data and acquire information needed to make pest management decisions. Automatic insect monitoring using acoustical sensors will be investigated.

Investigators: David W. Hagstrum, Paul W. Flinn, James E. Throne, Charles S. Burks

1. Specific Objective: Quantify effects of temperature and moisture on fecundity of maize weevils for inclusion in maize weevil model.

Progress FY96: Continued to work to develop a quick method for quantifying number of maize weevil egg in grain using immunological methods. Determined that polyclonal antibody that was developed for maize weevil egg plugs is too general. The antibody reacts with egg plugs, but will also react with grain that has had weevils on it but contains no egg plugs. Further purification of egg plug proteins to develop a more specific antibody is in progress.

Plan of Work FY97: Continue development of immunological method.

2. Specific Objective: Develop computer model for simulating population dynamics of the predator *Lyctocoris campestris*.

Progress FY96: Model was developed in conjunction with Megha Parajulee (Texas A & M Univ.) and Tom Phillips (OK State Univ.). Validation tests were run and model is being modified.

Plan of Work FY97: Modify model to improve simulation accuracy.

3. Specific Objective: Identify physiological factors involved in cold hardiness and acclimation at different temperature ranges.

Progress FY96: Obtained supercooling point data to differentiate chilling and freezing injury at sub-0°C temperatures. Developed metabolite assays to compare utilization of nutrient stores at temperatures below and above 0°C.

Plan of Work FY97: Methods developed in mosquitoes are being adapted to lesser grain borer. Rates of glycogen and lipid depletion will be determined in the lesser grain borer during chilling injury at various temperatures. These will be compared to depletion rates in more cold-tolerant stored grain product pests such as the red flour beetle and the rusty grain beetle. This knowledge is important because it seems likely that: a) Insects under aerobic stress, such as chilling or exposure to fumigants, depend heavily on glycogen reserves for their respiratory requirements, and b) if this is so, then quantifying glycogen reserves and rate of depletion can both aid modeling insect survival and suggest ways to synergize aeration and fumigation control strategies.

4. Specific Objective: Determine overwintering stages and cold tolerance of parasitoid wasps which attack stored grain pests, and find conditions lethal to the stored grain pest (i.e., the host) but not to its parasite.

Progress FY96: Obtained supercooling points and two-hour lower lethal threshold for the species *Anisopteromalus calandrae, Cephalonomia tarsalis,* and *Choetospila elegans.* Found that the species *Cephalonomia waterstoni* is very resistant to cold, but did not adequately distinguish chilling and freezing injury.

[illegible] — development of immunological method.

[illegible] 19 Objective: Develop computer model for simulating population dynamics of the [illegible] Lyctocoris campestris.

Progress, FY96: Model was developed in conjunction with Megha Parajulee (Texas A & M Univ.) and Tom Phillips (OK State Univ.). Validation tests were [illegible] and model is being modified.

[illegible] FY97: Modify model to improve simulation accuracy.

[illegible] Objective: Identify physiological factors involved in cold hardiness and [illegible] at different temperature ranges.

Progress, [illegible]: Obtained supercooling point data to determine chilling and freezing injury at sub-0°C temperatures. Developed metabolite assays to compare utilization of nutrient stores at temperatures below and above 0°C.

Plan of [illegible] FY97: Method developed to measure [illegible] are being adapted to [illegible] have [illegible]. Rates of glycogen and lipid depletion will be determined in [illegible] during chilling injury at various temperatures. These will [illegible] depletion rates in more cold-tolerant stored [illegible] product pests [illegible] beetle and the rusty grain beetle. This knowledge is [illegible] because it seems likely that: a) insects under aerobic stress, such as [illegible] exposure to fumigants, depend heavily on glycogen reserves for their [illegible] requirements, and b) if this is so, then quantifying glycogen reserves [illegible] depletion [illegible] both and modeling insect survival and suggest ways to [illegible] and [illegible] mitigation control strategies.

[illegible] Objective: Determine overwintering stages and cold tolerance of [illegible] which attack stored grain pests, and find conditions lethal to [illegible] grain pest (i.e., the host) [illegible] not to its parasite.

[illegible] FY96: Obtained supercooling points and two-hour lower lethal [illegible] Anisopteromalus calandrae, Cephalonomia tarsalis, [illegible]. Found that the species Cephalonomia waterstoni is [illegible] the [illegible] [illegible] chilling and freezing

Plan of Work FY97: Work is underway to determine cold conditions which induce delayed mortality in the rice weevil. This delayed mortality means that an animal which is chilled as a larva successfully completes larval development and molts to a pupa, but is subsequently unable to complete adult development. This may provide a way to release parasitoid wasps as larvae without augmenting a pest population. Work is also underway to characterize the time adult parasitoids (above species) are able to tolerate various temperatures. The objective of this work is to determine which stages are most cold tolerant, and what storage conditions these species could tolerate during winter or following a forced chilling treatment of stored grain.

5. Specific Objective: Evaluation of accuracy of predictions of lesser grain borer population growth model.

Progress FY96: Ideally, insects are detected early and predictive models are used to forecast when pest infestations will be severe enough that control will be needed. These models must be validated over the range of environmental conditions at which they will be used. The growth of lesser grain borer populations infesting stored wheat was monitored over a broad range of grain moisture and temperature conditions and data were used to check predictions of a published lesser grain borer population growth model. Over this range of environmental conditions, a published population growth model explained 64--96% of the variation in insect density. New data on lesser grain borer developmental time, reproduction and survival were used to improve population growth model predictions. Substituting new equations and mortality rate in the published model improved predictions, increasing by 3--24% the percentage of variation explained. The biggest improvements were at the most extreme grain moisture and temperature conditions. The new model will allow stored grain managers to reliably predict insect infestation levels over a broader range of environmental conditions.

Plan of Work FY97: Field studies will be conducted to increase our understanding of the phenology of infestation of newly harvested wheat.

6. Specific Objective: Modeling movement of red flour beetle along temperature gradient in stored grain.

Progress FY96: Predictions of insect population growth as a function of temperature can be valuable in making pest management decisions. Insect population growth in stored wheat during the fall and spring depends upon their location in the grain mass because temperature gradients of as much as 7-10°C

[illegible] FY88: Work is underway to determine cold conditions which [illegible] in the rice weevil. This delayed mortality means that an animal which is chilled as a larva successfully completes larval development and molts [illegible], but is subsequently unable to complete adult development. [illegible] may provide a way to reduce parasitoid wasps as larvae without [illegible] a [illegible] population. Work is also underway to characterize the [illegible] parasitoids (above species) are able to tolerate various temperatures. The [illegible] is to determine which stages are most [illegible] to expose, and [illegible] conditions these species could tolerate during winter or following [illegible] chilling treatment of stored grain.

5. Specific objective: Evaluation of accuracy of predictions of lesser grain borer population growth model.

Progress FY88: Ideally, insects are detected early and predictions of [illegible] are used to determine when insect infestations will become large enough that control will be [illegible]. These models must be validated over the range of environmental conditions at which they will be used. The growth of lesser grain borer populations infesting stored wheat was monitored over a broad range of grain [illegible] and temperature conditions and data were used to check predictions of [illegible] lesser grain borer population growth model. Over this range [illegible] conditions, a published population growth model explained [illegible] variation in insect density. New data on lesser grain borer [illegible] time, reproduction and survival were used to improve population [illegible] predictions. Substituting new equations and mortality rate in the published model improved predictions, increasing [illegible] the percentage of [illegible] explained. The biggest improvements were at the more extreme grain [illegible] and temperature conditions. The new model will allow stored grain [illegible] to more accurately predict insect infestation levels over a broader range of [illegible].

[illegible] These studies will be conducted to increase our [illegible] of the phenology of infestation of newly harvested wheat.

[illegible] Modeling movement of red flour beetle along [illegible] in stored grain.

[illegible] of [illegible] population growth as a function of [illegible] decisions. Insect [illegible] and grain operators often [illegible]

per meter occur in stored wheat as grain cools or warms, respectively, from the outside to the center. Accurate prediction of population growth rates will therefore require more information on insect movement and their temperature preferences. The response of adult red flour beetles to a 14°C temperature gradient in a 7.6 x 7.6 x 61 cm mass of wheat was studied using 8 microphones to monitor the distances moved over time and temperature preference. Males tended to disperse more readily than females but both preferred temperatures >30°C. Single adults spent more time at the warm end than at the cold end of the temperature gradient. The temperature preference was much more evident with groups of 6 adults than with single adults. The presence of other adults apparently increased the time that adults spent at preferred temperatures. Observations on the times spent at different temperatures and the distances moved are a first step in developing a predictive model for insect movement in stored grain. An insect movement and temperature preference model should improve our predictions of temperature-dependent insect population growth.

Plans of Work FY97: Similar studies will be conducted with rice weevils and lesser grain borers.

7. Specific Objective: Develop computer model to predict the effectiveness of low oxygen atmosphere for rice weevil control.

Progress FY96: A spatial model of rice weevil population dynamics and bin temperature was used to predict the efficacy of low oxygen atmosphere to suppress insect pests in bins of stored wheat. Equations were incorporated into the model that predict the effects of low oxygen atmosphere, grain temperature, and insect stage on insect mortality. This model can predict the duration of fumigation required to produce a given mortality using low oxygen levels. It can also be used to predict insect density in grain 1-2 months post-fumigation. Simulations showed that fumigating grain at 29°C for 15 days resulted in 99.9% mortality to all stages of rice weevil. However, fumigating 17°C grain for 15 days only resulted in 95.7% mortality of rice weevil pupae. Thus, cool grain near the bin walls required longer fumigation intervals to kill all insect stages, and the pupal and adult stages of rice weevil took longer to kill than the egg and larval stages. The model will be useful in comparing different control methods prior to deciding which to use.

Plan of Work FY97: The model will be incorporated in the Stored Grain Advisor, an expert system for stored-grain management.

Publications:

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... J. E., D. K. Weaver, V. Chew, and J. ... Baker. 1995. Probit analysis of correlated data: multiple observations over time at one concentration. J. Econ. Entomol. 88: 1510-1512.

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... Throne, ... J. E. Baker, and V. Chew. 1995. Potential probit analyses for ... assessment of insecticide resistance. Resis. Pest ... Newsletter 7: 20-21. [Abstract]

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-015-00D

Title: Biological Control of Stored Product Insects With Parasites, Predators and Entomopathogens

Main Objectives:

The goal of this project is to develop economically viable pest management systems that capitalize on desirable biochemical, behavioral and biological traits of parasites, predators and entomopathogens of stored grain insect pests. Specific objectives include characterizing life histories of promising parasites and predators exposed singly and in combination to single- and multiple-species combinations of stored product pests; identifying the semiochemical and behavioral mechanisms used by these biological control agents to locate, recognize and kill their hosts; characterizing behavioral and semiochemical responses by pests that ameliorate the effectiveness of biological control agents; assessing specific stress factors such as temperature, relative humidity, desiccants and inhibitors of insect-specific hormones that might weaken pests and make them more susceptible to biocontrol agents; determining parasite-host release ratios, timing of releases, and effect of multiple-species releases on control efficacy; evaluating possible detrimental effects of releasing biological control agents into stored grain commodities; elucidating the biochemical mechanisms by which the toxins of *Bacillus thuringiensis* (*B.t.*) kill stored product pests, with particular emphasis on the physiology of membrane receptor-protein interactions; evaluating the extent of resistance and cross-resistance to native, cloned, and truncated *B.t.* toxins in stored product insect populations; and characterizing insect resistance mechanisms involving the interaction of *B.t.* toxin proteins with midgut proteinases in *Plodia interpunctella*.

Investigators: Ralph W. Howard, Donovan E. Johnson, William H. McGaughey, Brenda Oppert

1. Specific objective: Identify cuticular hydrocarbons from the parasite *Cephalonomia stephanoderis* and its beetle host.

Progress FY96: Hydrocarbons were identified and compared to the stored product parasite *C. waterstoni* and its beetle host, whose hydrocarbons we earlier had identified. Substantial differences were found between the two

parasites and between the two hosts, but both showed strong sex-related differences that suggest that their hydrocarbons are being used for species and gender recognition. Analysis completed and work reported in the Annals of the Entomological Society of America.

Plan of Work FY97: This study has been completed.

2. Specific Objective: Determine the mechanisms by which *Cephalonomia waterstoni* and *C. tarsalis* recognize their specific hosts, the rusty grain beetle and the saw-toothed grain beetle, respectively.

Progress for FY96: Cultures of both parasites and their hosts were established. Protocols for conducting detailed video behavioral analyses were established. Conducted detailed behavioral analyses and generated an ethogram for host recognition by *C. tarsalis*.

Plan of Work FY97: Continue with development of ethograms for *C. waterstoni*. Publish the initial results of the study with *C. tarsalis*, and continue development of bioassays for chemical cues used in host recognition.

3. Specific Objective: Evaluate the possible roles of pheromones in sexual behavior by the parasitoids *Cephalonomia tarsalis* and *Anisopteromalus calandrae.*

Progress for FY96: The parasites and their hosts were established in cultures. Video protocols were established for generating ethograms and preliminary behavioral data were gathered.

Plan of Work FY97: Conduct detailed behavioral analyses of the courtship and copulatory behavior of the parasites. Using the resulting ethograms, develop bioassays to separate chemical from other cues. Begin isolation of possible pheromones.

4. Specific Objective: Characterize the cuticular hydrocarbons of the parasitoid *Pteromalus cereallallae* and determine their semiochemical functions.

Progress for FY96: Hydrocarbons have been isolated from male and female parasitoids and percent compositions calculated. Determination of the structures of the hydrocarbons is still in progress. There are striking gender based differences between the males and females.

[illegible] [illegible] the two hosts, but both showed strong sex-related [illegible] that suggest that their [illegible] are being used for species and [illegible]. Analysis completed and work reported in the Annals of the [illegible] Society of America.

[illegible] of Work FY97: This study has been completed.

[illegible] Objective: Determine the [illegible] by which *Cephalonomia* [illegible] and *C. tarsalis* recognize their specific hosts, the rusty grain [illegible] [illegible] grain beetle, respectively.

[illegible] FY96: Cultures of both parasites and their hosts were established. Protocols [illegible] conducting detailed video behavioral analyses were established. [illegible] behavioral analyses were generated an ethogram for host recognition by *C. tarsalis*.

Plan of Work FY97: Continue with development of ethograms [illegible] *C.* [illegible]. Publish the initial results of the study with *C. tarsalis* and continue [illegible] of bioassays for chemical cues used in host recognition.

Specific Objective: Evaluate the possible roles of pheromones [illegible] [illegible] by the parasitoids *Cephalonomia tarsalis* and *Anisopteromalus calandrae*.

[illegible] FY96: The parasites and their hosts were established in cultures. [illegible] protocols were established for generating ethograms and preliminary [illegible] data were [illegible].

[illegible] Work [illegible] Conduct detailed behavioral analyses of the courtship and [illegible] of the parasites. Using the resulting ethograms, develop [illegible] chemical [illegible] other cues. Begin isolation of possible

Plan of Work FY97: Complete identification of all hydrocarbons. Complete statistical analyses of compositional differences, and develop bioassays to characterize semiochemical functions of the hydrocarbons.

5. Specific Objective: Identify cues by which parasitoids locate grain stores containing stored product insects.

Progress for FY96: The wind tunnel bioassay chamber was tested in preliminary experiments using the parasite *C. tarsalis* and its host, the saw-toothed grain beetle.

Plan of Work FY97: Construct a wind tunnel bioassay chamber specific for parasitoids and begin experiments with four stored product parasitoids, assessing their orientation behavior towards odor volatiles from stored grain and stored grain infested with storage pests.

6. Specific Objective: Assess the host-specificity and control efficacy of *Pteromalus cerealellae* when exposed to low-level populations of multiple hosts in stored wheat.

Progress for FY96: Cultures of all insects were established and initial tests have begun.

Plan of Work FY97: Continue experiments, analyze results, and publish the findings.

7. Specific objective: Investigate the involvement of insect gut proteinases in the development of resistance to insecticidal toxins from *Bacillus thuringiensis* (B.t.).

Progress FY96: Previously, strains of the Indianmeal moth, *Plodia interpunctella* were analyzed for overall gut proteinase activities, using general and specific enzyme substrates and inhibitors. The major gut activities were found to be serine proteinases, with predominately trypsin and chymotrypsin-like activities. Class-specific inhibitors used in a protoxin assay indicated that chymotrypsin-like enzymes were important in protoxin hydrolysis, with trypsin-like enzymes also involved to a lesser degree. The results of this study were published.

B.t. susceptible and resistant strains of *P. interpunctella* were analyzed for specific proteinase activities, and it was discovered that some of the B.t. resistant strains lack a critical gut proteinase. Genetic crosses were initiated in order to link the absence of this proteinase to B.t. resistance. The results of this study indicate a genetic linkage, and a summary of the study is being prepared for publication.

Plan of Work FY97: Our research continues to focus on the lack of proteinase activity in some B.t. resistant strains and its relationship to toxin resistance, using molecular biology, microscopy, and biochemical techniques.

8. Specific Objective. Determine effects of spores on entomocidal activity of CryIAb and CryIC crystal proteins from *Bacillus thuringiensis* toward the Indianmeal moth.

Progress FY96: The toxicities of spore-crystal powders were related to relative crystal protein content as well as viable spore count. Spore/crystal ratio, protein contribution from spores and viable spore count all contributed to the overall potency of individual *B. thuringiensis* preparations toward the Indianmeal moth.

Plan of Work FY97: Proteins that bind to Cry toxin binding sites are also present in spore coats of certain *B. thuringiensis* species. Determine if membrane binding plays a role in spore germination and/or influences *B. thuringiensis* septicemia in susceptible and resistant Indianmeal moth larvae.

9. Specific Objective. Determine membrane binding characteristics of susceptible and *B. thuringiensis* resistant Indianmeal moth for crystal proteins from *B. thuringiensis*.

Progress FY96: Two procedures were discovered that affect the level of detection of binding proteins in brush border membrane vesicles (BBMV). One is the method of membrane disruption; a non-ionic detergent conserves micelle formation and leads to improved toxin protein binding, and the second involves exposure of BBMV to toxin proteins prior to disruption of the membrane vesicles that seem to improve specific protein binding.

Plan of Work FY97: Compare membrane binding proteins from susceptible and resistant Indianmeal moth larvae to specific Cry toxin proteins. Can resistance to a particular Cry toxin protein be correlated with the presence or absence of a specific binding protein?

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Oppert, B. S., K. J. Kramer, and W. H. McGaughey. Examination of differences in *Plodia interpunctella* gut proteolytic activity using activity blots. Presented at the 128th Annual Meeting of the Kansas Academy of Sciences, Emporia, KS., March 1995. (Abstract)

Oppert, B. S., K. J. Kramer, and W. H. McGaughey. *Bacillus thuringiensis* resistant and susceptible strains of *Plodia interpunctella* have differences in trypsin-like gut proteinases. Presented at the National Forum on Insect Resistance to *Bacillus thuringiensis,* Washington, DC, April 1996. (Abstract)

Zhu, Y. C., B. S. Oppert, A. K. Dowdy, and W. H. McGaughey. Sequence and expression of gut proteinase genes from *Plodia interpunctella.* Presented at the National Entomological Society of America Annual meeting, Las Vegas, NV, December 1995. (Abstract)

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-016-00D

TITLE: Development of Physiological and Genetic Controls for Stored Product Insects

Main Objectives:

The goals of this project are to characterize physiological and genetic processes that can be manipulated for insect control purposes, identify inhibitory proteins and genetically modify cereal grains and entomopathogens with their genes, develop techniques for genetic manipulation of insects, devise genetic methods for managing pesticide resistance in insects and for enhancing the efficacy of beneficial insects. Specific objectives include identifying target sites in insect skeletal, gut and endocrine systems vulnerable to biopesticides; characterizing molting and digestive enzymes, identifying inhibitors whose genes are amenable to plant and microbial genetic engineering; evaluating recombinant seeds for resistance to insects and evaluating recombinant microbial pathogens for efficacy as biological control agents; identifying inhibitors of insect hormones, evaluating modes of action and efficacy as regulators of growth and physiological processes; characterizing cuticular components of pests and parasites, assessing functional responses to environmental stress; characterizing genetic mechanisms regulating reproduction, development and pesticide resistance of pest and beneficial insects; and developing techniques for genetically altering, manipulating and monitoring populations of pest and beneficial insects.

Investigators: Karl J. Kramer, James E. Baker, Richard W. Beeman, Ralph W. Howard

1. Specific Objectives: Identify potential biopesticides and screen them for their ability to disrupt stored grain insect development and physiology.

Progress FY96: Amylase inhibitors from wheat were screened for insect growth inhibiting activity. Several were inhibitory against beetle species. Two reviews on biopesticides for insect pest management were written. Projuvenoid insect growth regulators were screened for activity toward stored grain insect pests. Several were active against weevil species. A review on the use of proteinase inhibitors as biopesticides was written.

Plan of Work FY97-98: Continue studies on the development of biopesticides and insect growth regulators that disrupt insect gut and cuticle physiology for use against insect pests in food and stored products.

2. Specific objectives: Characterize reactants, intermediates, products and enzymes involved in insect cuticle sclerotization, which might be disrupted by novel insect-selective control agents.

Progress FY96: A review on the use of solid state nuclear magnetic resonance for studies of insect sclerotized structures was written. Several enzymes, metabolites, proteins, and cross-linked structures responsible for cuticle sclerotization were characterized. Mechanisms for reactions between cuticle cross-linking agents and protein model nucleophiles were elucidated.

Plan of Work FY97-98: Continue to identify and characterize insect-specific reactions that occur during cuticle sclerotization which could serve as targets for new insect growth regulators and biopesticides for use in protecting food and stored products against insect pests.

3. Specific Objective: Clone insect chitinolytic enzyme genes and study their molecular biology to determine how insects regulate chitin degradation during molting. Use chitinolytic enzymes as biopesticides to help control pest insects by manipulating chitinolytic enzyme genes in transgenic plants and microbial entomopathogens.

Progress FY96: Characterization of a genomic clone and a cDNA for insect chitinase and a β-N-acetylglucosaminidase, respectively, was completed. A review on the use of chitinases as biopesticides for insect control was written. The properties of a recombinant insect chitinase isolated from transgenic tobacco were determined. A foreign patent application for recombinant insect chitinase and its use as a biocide was submitted.

Plan of Work FY97-98: Continue to develop insect chitinolytic enzymes as biopesticides for use in protecting food and stored products against insect pests and microbial pathogens.

4. Specific Objective: Determine genetic basis of malathion resistance in the pteromalid parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae).

Progress FY96: The genetic basis of malathion resistance in the parasitic wasp *A. calandrae* was determined by appropriate reciprocal F_1 hybrid crosses, backcrosses, and inter se (self crosses) between the resistant strain and a susceptible laboratory strain. Results of bioassays of progeny from each type of cross indicate that the resistance in this haplo-diploid parasitoid is inherited as an incompletely dominant trait controlled by a single gene.

Plan of Work FY97: Prepare manuscript describing research results.

5. Specific Objective: Compare fitness parameters of resistant and susceptible strains of *A. calandrae* parasitizing rice weevil larvae in wheat.

Progress FY96: Studies on fitness of resistance alleles in *A. calandrae* indicate no dramatic differences in fecundity, development time of single cohorts, or parasitization efficiency between the resistant and susceptible strains when the wasps are parasitizing rice weevil larvae in hard, red winter wheat. However, in some tests, fecundity of the laboratory (susceptible) strain was slightly but significantly higher than that of the resistant strain. In addition, the progeny sex ratio of the laboratory strain was more biased towards more female progeny than that of the resistant strain when host density was increased. As part of the fitness study, frequency of R alleles was monitored in Hardy-Weinberg populations. The R alleles were competitive with S alleles during 6 generations.

Plan of Work FY97: Continue studies to characterize fitness of insecticide-resistant *A. calandrae.*

6. Specific Objective: Initiate studies to characterize the biochemical mechanism(s) of malathion resistance in the parasitoid *A. calandrae.*

Progress FY96: We have begun a comparative study on carboxylesterase activity in the resistant and susceptible strains of *A. calandrae*. Preliminary kinetic studies with α-napthol acetate as substrate indicate that activity (vmax) in the soluble fraction of homogenates of female adults of the resistant strain was 1.6 fold higher compared with that of susceptible females. Additional substrates are being analyzed.

Plan of Work FY97: Continue to characterize the biochemical mechanisms of resistance in *A. calandrae.*

7. Specific Objective: Characterize molecular genetics of malathion resistance in *A. calandrae.*

Progress FY96: cDNA libraries of adults of resistant and susceptible strains of *A. calandrae* have been prepared. The libraries have been screened with selected primers. Several positive clones have been obtained.

Plan of Work FY97: Continue the analysis of molecular mechanisms of malathion resistance in the hymenopterous parasitoid *A. calandrae.*

8. Specific Objective: Develop methodology for genetic manipulation of pest insects.

Progress FY96: We tested a lepidopteran transposable element for ability to function as a gene transfer agent in flour beetles. Several putative transformants were obtained. Collaborators include Malcom Fraser (Notre Dame Univ.), Paul Shirk (ARS, Gainesville, FL), and Rob Denell (Kansas State Univ.).

Plan of Work FY97: Work is underway to confirm the putative transgene lines, and to continue to test other candidate gene transfer vectors. Expected benefits include the ability to infect pest species with insect control genes (viruses, lethal genes, transposons), transfer of pesticide resistance genes to parasitoids, and transfer of deleterious traits (such as disease susceptibility, pesticide susceptibility and cold intolerance) to pest species.

9. Specific Objective: Characterize insect resistance to grain protectants.

Progress FY96: A gene for pyrethroid resistance was found to be associated with a gene for cytochrome P450. The P450 gene is overexpressed in resistant insects, and may be directly responsible for the resistance.

Plan of Work FY97: Molecular genetic characterization of the gene is in progress. Collaborators: Jeff Stuart (Purdue Univ.) and Richard ffrench-Constant (Univ. Of Wisconsin-Madison).

10. Specific Objective: Harness natural insect control genes.

Progress FY96: Map-based cloning of a naturally-occurring killer gene was initiated. A whole-genome molecular map was constructed, and DNA markers closely linked to the killer gene were identified.

Plan of Work FY97: We will continue to pursue the map-based cloning of the killer gene. In FY97 we plan to clone additional DNA markers closely linked to the gene, and to develop strategies for physical mapping in the immediate vicinity of the gene.

11. Specific Objective: Evaluate the capability of the tenebrionid beetle model stored-product pest *Zophobas atratus* to produce nodules when challenged with a bacterial infection, and determine if nodules formation is regulated by eicosanoids.

Progress FY96: *Z. atratus* larvae of all ages were shown to produce nodules when infected with the pathogenic bacterium, *Serratia marcescens*. The fatty acid composition of a major immune tissue, the fat body, and the major excretory organ, the Malpighian tubules, were shown to contain the necessary precursor fatty acids for producing eicosanoids in larvae, pupae, and adults.

Plan of Work FY97: Begin studies to assess which eicosanoids are involved in the nodulation process in this insect.

12. Specific Objective: Evaluate the changes in cuticular hydrocarbon composition of larvae and adults of the saw-toothed grain beetle when exposed to low temperatures or dessicants.

Progress FY96: Factorial experiments were conducted to obtain response surfaces for changes in hydrocarbon profiles of the saw-toothed grain beetle with changes in temperature.

Plan of Work FY97: Complete statistical analyses and publish results.

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Baker, J. E. and K. J. Kramer. 1996. Biotechnological approaches for stored-product insect pest control. Postharvest News and Information 7, 11N-18N.

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RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-017-00D

Title: Monitoring and control strategies for stored-product insects

Main objectives:

The overall objective of this CRIS is to develop monitoring and control strategies for stored-product insect pests in and around storage and processing facilities. These studies will lead to the development and improvement of genetic, semiochemical and physical techniques to monitor insect behavior, estimate population densities, and determine control thresholds and timing of management actions. Selected control tactics will be evaluated for integration into ecologically-compatible pest management programs. Insect resistant packaging and barriers will be developed to prevent infestation of stored products. The acquired knowledge will lay the ground work necessary to evaluate the efficacy of current practices and to develop ecologically-sound insect pest management programs.

Investigators: Michael A. Mullen, Franklin H. Arthur, Alan K. Dowdy, Yu-Cheng Zhu

1. Specific Objective: Development of a disposable pheromone baited trap for stored-product Coleoptera.

Progress FY96: The trap was made from a 15 dram snap cap vial with openings for the insects to enter and it was baited with a pheromone lure. A food oil was used as a short range attractant and a killing agent. Laboratory comparisons with the FLIT TRAK M^2 that showed the vial trap to be about equal in effectiveness for capturing adult red flour beetles. This trap was designed to be used in areas where the FLIT TRAK^2 would not be suitable. Research has been completed and a manuscript has been submitted to J. Stored-Products Research.

Plan of Work for FY97: The trap will be included in a study in a grain processing plant.

2. Specific Objective: Evaluation of pheromone formulations for the saw-toothed grain beetle.

Progress FY96: No new pheromone formulations were received and the project is currently on an indefinite hold.

Plan of Work FY97: None planned.

3. Specific Objective: Redesign the FLIT-TRAK M^2 to improve the rate of catch and ease of counting trapped insects.

Progress FY96: A redesign of the trap was initiated and testing of several prototypes was started. The emphasis is on developing a trap with a lower profile and a one piece design to make the trap easier to use. Recommendations were made to the design engineer to alter the design without impacting its effectiveness.

Plan of Work FY97: As new prototypes become available testing will be continued.

4. Specific Objective: A new pheromone trap will be designed for use in specialized areas, such as grocery stores, so that they will remain out of the view of the public.

Plan of Work FY97: Trap designs will be tested in grocery stores and warehouses for their effectiveness for the Indianmeal moth, as well as several stored-product beetles.

5. Specific Objective: Use of pheromone traps as a dispersal mechanism for granulosis virus.

Progress FY96: Initial studies showed that virus dispersal using pheromone traps is not very effective.

Plan of Work FY97: None planned.

6. Specific Objective: Improvement of seals and closures on commercial packaging to reduce infestation by stored-product insects.

Progress FY96: Cooperation with food processing companies was continued. Tests for several companies were conducted. Improved packages for dry dog food to be exported to Japan were completed. Several cartons for pancake mix were tested and the best designs will be used for export to Latin America. Other studies were conducted on improved packaging for dry pet foods, breakfast cereals, and baby food.

Plan of Work FY97: Testing of packages will be continued and recommendations will be made to the manufacturers to improve insect resistance.

7. Specific Objective: Test chemical odor neutralizers for use in insect resistant packaging.

Progress FY96: The bioassay developed was used to test the odor neutralizer. Preliminary tests were initiated to determine the effectiveness of the material.

Plan of Work FY97: Testing of the material will continue. In cooperation with the manufacturer, this material will be incorporated into different packaging materials to determine its usefulness in commercial packaging.

8. Specific Objective: Test, evaluate, and make recommendations on several new repellent compounds for use in insect resistant packaging.

Progress FY96: Tests were set up in September to test these compounds. Initial tests are being conducted on dry cat food and baby cereal.

Plan of Work FY97: The first round of tests will be concluded and an evaluation of the compounds will be made. Further testing will be conducted on those compounds with some promise.

9. Specific Objective: Examine sublethal effects of high temperature on insect population structure. Consider efficacy of heat as an alternative to methyl bromide fumigation.

Progress FY96: Laboratory tests indicated that there is little difference in mortality caused by high temperature between confused flour beetles and red flour beetles.

Plan of Work FY97: Work has been initiated to examine the effects of sublethal temperatures on the reproductive capabilities of flour beetles. Collaborative work will be established with a national food processor who uses heat as an alternative to methyl bromide.

10. Specific Objective: Precision targeting of insect infestations in commercial storage and retail facilities.

Progress FY96: The objective of this work is to improve insect monitoring and detection programs in processing and warehouse facilities. Insect monitoring was conducted in five grocery stores, two warehouses, and one processing plant. Insects were detected in all facilities examined and population size appears to be related to basic sanitation practices. Fewest insects were detected in the cleanest facilities.

Plan of Work FY97: Work will continue on development of precision targeting methods for processing plants, warehouses, and grocery stores.

11. Specific Objective: Evaluate the use of multiple insect pheromones in single traps.

Progress FY96: The possibility of using more than one insect pheromone in a trap was tested. The species tested included lesser grain borers, red flour beetles, and warehouse beetles. Only slight negative effects occurred when using pheromone lures for more than one insect in a single trap. Thus, separate traps are not necessary for detecting these species in warehouses and processing plants. This will result in reduced costs associated with purchasing traps and reduced labor in maintaining an efficient pest surveillance program.

Plan of Work FY97: No additional work is currently planned.

12. Specific Objective: Determine sources of insect infestation along marketing channels using DNA fingerprinting technology.

Progress FY96: Indianmeal moths collected from a warehouse in Illinois and a retail store in California were examined to determine if they originated from different sources. No differences in the DNA fingerprint were detected after analysis with 56 genetic primers.

Plan of Work FY97: No additional work is currently planned.

13. Specific Objective: Examine DNA fingerprints to detect and monitor for insecticide resistance in field populations.

Progress FY96: Characterizing the expression of messenger RNA that codes for trypsin, chymotrypsin and aminopeptidase in Indianmeal moths was conducted.

Plan of Work FY97: Research will continue on using DNA fingerprinting to detect and monitor insecticide resistance. This may eventually lead to a quick test to be used in field situations.

14. Specific Objective: Evaluation of residual insecticides on different flooring surfaces.

Progress FY96: A 0.05%AI deltamethrin dust formulation that is labeled as a crack and crevice treatment was evaluated as a residual treatment on plywood, tile, and concrete surfaces. The maximum label rate of 3 grams per square yard controlled red flour beetles and lesser grain borers on all three surfaces for about 20 weeks. The confused flour beetle was more tolerant than the other two species. The dust did not control confused flour beetles on plywood, but did control this beetle on tile and concrete for about 9-10 weeks.

Plan of Work FY 97: Cooperate with industry to conduct evaluations of new chemicals. Conduct additional research with established chemicals.

15. Specific Objective: Determine residual efficacy of cyfluthrin and deltamethrin as grain protectants.

Progress FY96: Timed exposure studies have been initiated for cyfluthrin. A new cyfluthrin formulation will be evaluated as a grain protectant.

Plan of Work FY97: Continue with experiments that are in progress.

16. Specific Objective: Determine the potential for the expanded use of aeration on stored grains.

Progress FY96: Data have been collected and models are being developed for the increased use of aeration to manage insect pests in stored corn and stored wheat. Aeration plans that incorporate insect growth models will be developed for specific geographic regions.

Plan of Work FY97: Continue cooperative efforts regarding insect population models and how they could be used to develop aeration plans.

17. Specific Objective: Evaluate microbial pathogens as protectants of stored grains.

Progress FY96: Cooperated with several companies to test microbial and fungal pathogens to control stored-product insects. Results have been inconclusive regarding efficacy and new testing methods must be developed.

Plan of Work FY97: Refine testing methodologies and continue with research regarding promising candidate microbial and fungal pathogens.

Publications:

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